Biofilms in chronic bacterial infections and Quorum sensing

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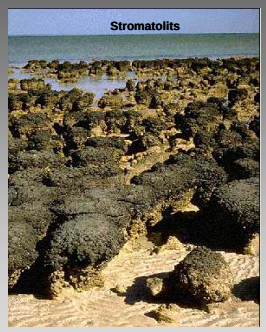
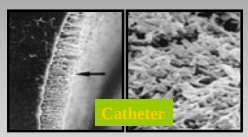
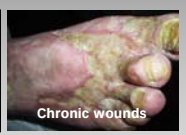
The most natural phenomenon

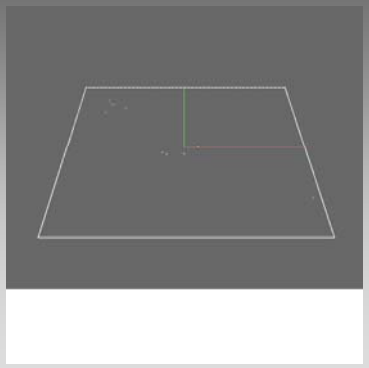


…but in the way of humans

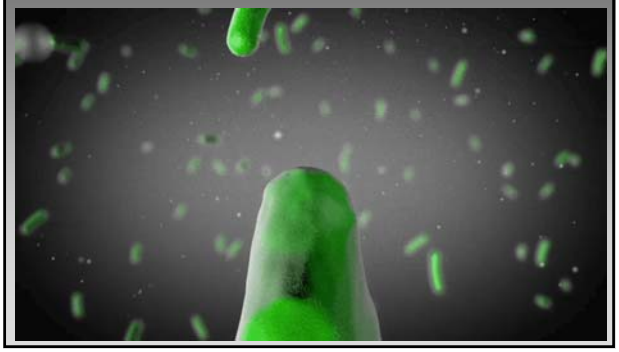




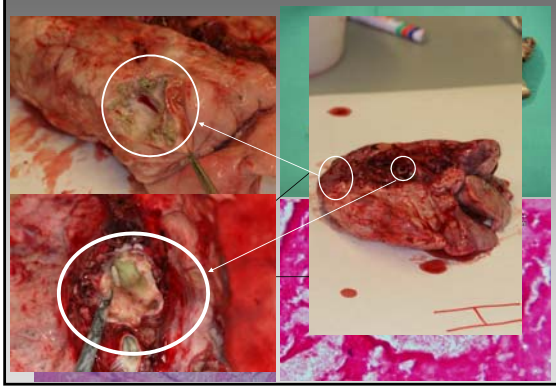
One on top of the other



The problem

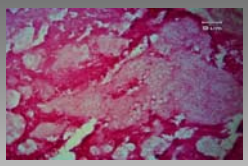


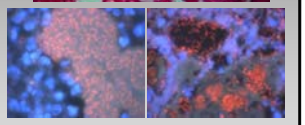
Cystic fibrosis – the classical example



Aggressive suppressive therapy

CF male
28 years of chronic PA infection
2 week anti PA treatments
20 years daily colistin/tobramycin inhalations





1 kg tobramycin,
10 kg beta-lactam anti-pseudomonas antibiotics
and 1 kg inhaled colistin

Bjarnsholt et al; *Pseudomonas aeruginosa* biofilms in the Respiratory Tract of Cystic Fibrosis Patients; Pediatr Pulmonol. 2009 Jun;44(6):547-58

A present problem:

Adverse reactions to polyacrylamide gel are seen as swellings or nodules, and controversy exists whether these are due to bacterial infection or an autoimmune reaction to the filler.

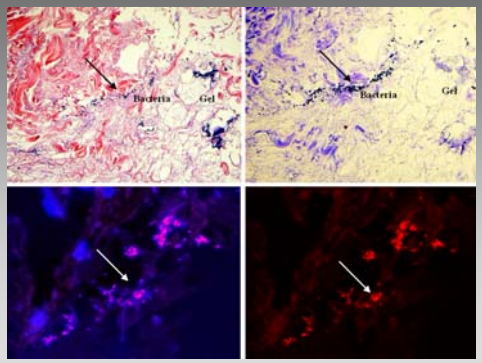
4 days after steroid

Courtesy of Lise Christensen

site	Type of PAAG	Time since inj	Initial treatment	Time with AE
Cheek	Aquamid	2 years	Steroid inj Later AB inj	7 months
Lip	Aquamid	½ year	Steroid inj Later ABs	½ year
Lip	Aquamid	1 month	Steroid + ABs Later ABs	2 years
Breast	Amazing gel	2 years	ABs liposuction	5 months
Tear-trough	Aquamid	2 years	Steroid inj Later ABs	½ year
Naso-labial fold	Aquamid	14 days	Steroid ABs+surgery	1½ year
Lip	Interfall gel	½ year	Steroid ABs+surgery	2½ years
Penis	Aquamid	2 years	Steroid AB inj+surgery	1½ years

Courtesy of Lise Christensen

Soft tissue fillers



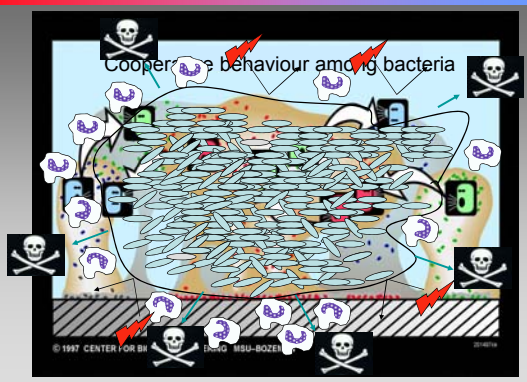
Bjarnsholt et al:; Dermatol.surg. 2009 (Aug)

Treatment

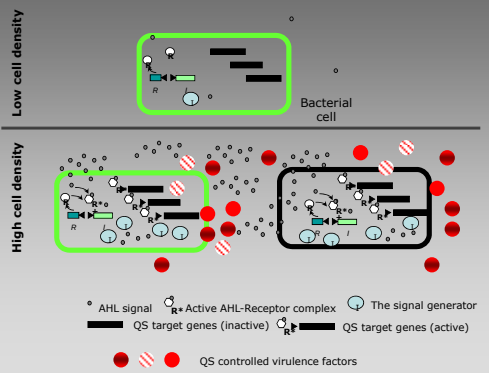
- (I) Early aggressive antibiotic treatment before the biofilm is formed
- (II) Chronic (rest of life) suppressive antibiotic treatment when biofilm have formed, if the infected area can not be removed

  – **User guide for KMA-Rigshospitalet**

Quorum sensing and Biofilms



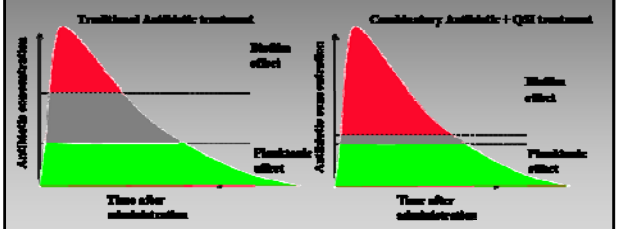
QS Communication



Attenuation of bacteria

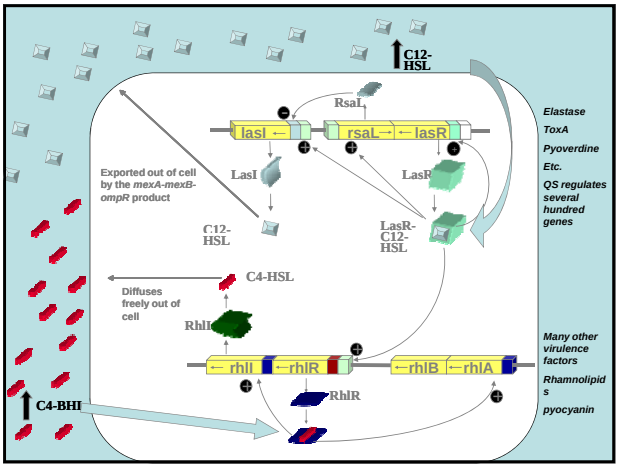
- Quorum sensing a new drug target
  - renders the biofilm susceptible by jamming the command language

QSI widens the therapeutic window

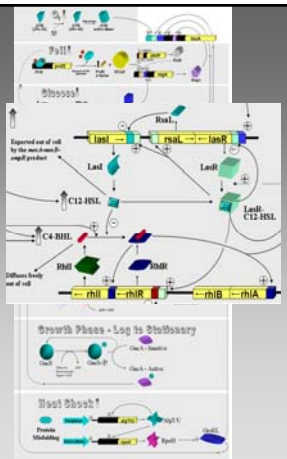


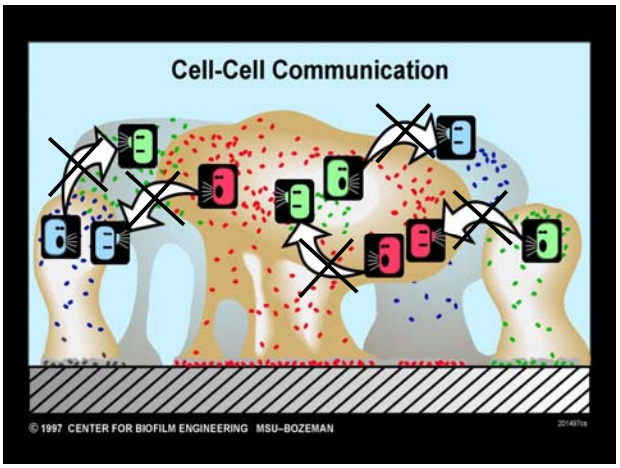
The opportunist *P. aeruginosa*

- 16% of nosocomial pneumonia cases
- 12% of hospital-acquired urinary tract infections
- 8% of surgical wound infections
- 80% of all large chronic wounds contain *P. aeruginosa*
- 10% of bloodstream infections
- 30% deaths in immunocompromised patients
- 38% deaths in intubated patients
- Associated with 60% of deaths under outbreaks in burn units
- Associated with 50% of deaths in the expanding AIDS population
- Cystic fibrosis patients are susceptible to a chronic pulmonary infection, which is responsible for high rates of illness and death
- CF is the most frequent severe genetic disease among Caucasians (1:4700 in Denmark)



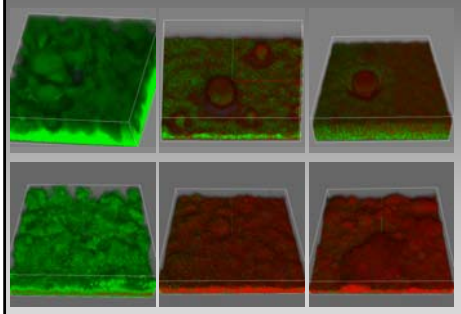
P. aeruginosa – quorum sensing in the grand scheme





Tobramycin sensitivity

0 γ tobra | 10 γ tobra | 20 γ tobra



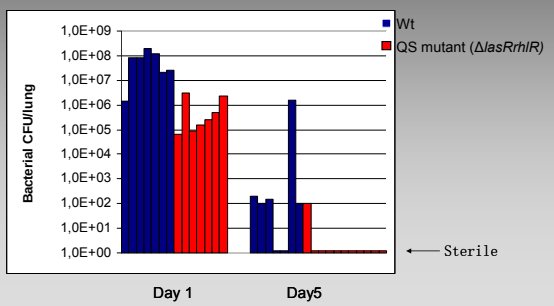
PAO1

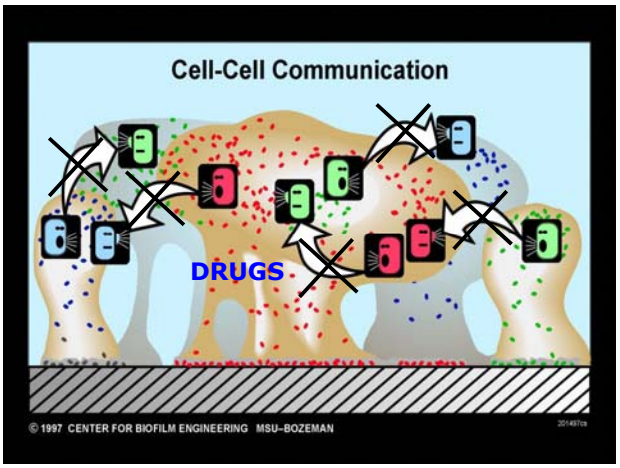
QS mutant (Δ*lasRrhlR*)

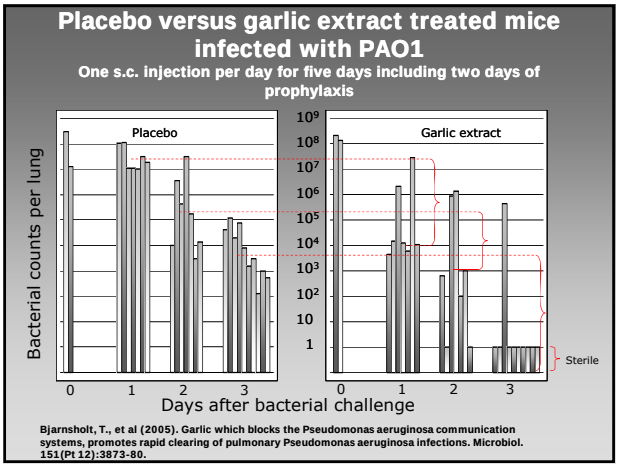
-3 days old *P. aeruginosa* Δ*lasRrhlR* biofilms
-The biofilms were exposed to **tobramycin** for 48 h
-Biofilm viability assayed using **LIVE/DEAD *Bac*Light Bacterial Viability Kit**.

PAO1 vs. Δ*lasR rhlR*

- Mortality
  - PAO1 – 73%
  - QS mutant (Δ*lasR rhlR*) – 46%

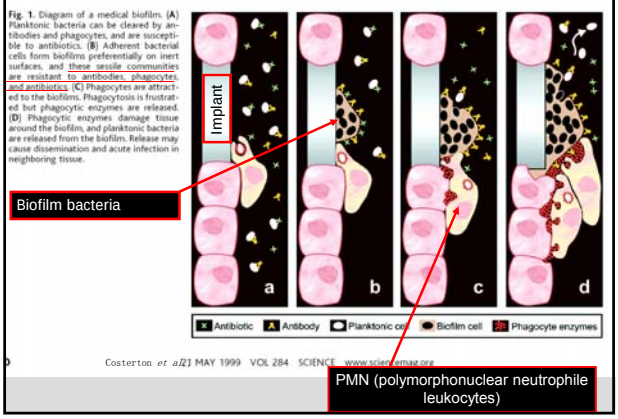



Cell-Cell Communication
DRUGS
© 1997 CENTER FOR BIOFILM ENGINEERING MSU-BOZEMAN

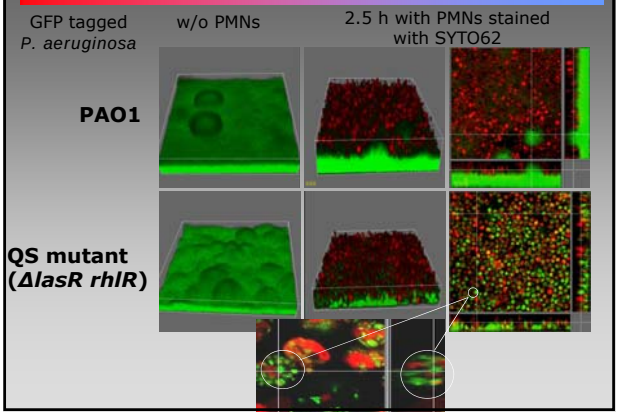

Placebo versus garlic extract treated mice infected with PAO1
One s.c. injection per day for five days including two days of prophylaxis
Placebo
Garlic extract
Bacterial counts per lung
10^9
10^8
10^7
10^6
10^5
10^4
10^3
10^2
10
1
Sterile
0 1 2 3
0 1 2 3
Days after bacterial challenge
Bjarnsholt, T., et al (2005). Garlic which blocks the Pseudomonas aeruginosa communication systems, promotes rapid clearing of pulmonary Pseudomonas aeruginosa infections. Microbiol. 151(Pt 12):3873-80.


QS blocker activities in natural products
Sample
QS blocker
Bean sprout +
Blackberry -
Brown onion -
Chamomile +
Carrot +
Coffee -
Cranberry -
Poison Ivy -
Garlic +
Gele Royal -
Ginseng -
Habanero +
Honey (various sorts) -
Leek -
Mint-tea -
Propolis +
Raspberry -
Red Chili -
Spring onion -
Tea Tree Oil -
Water Lilly +
Yellow pepper +
Blood (plasma) -
Stinging nettle -
Anemone -
Snowberry -

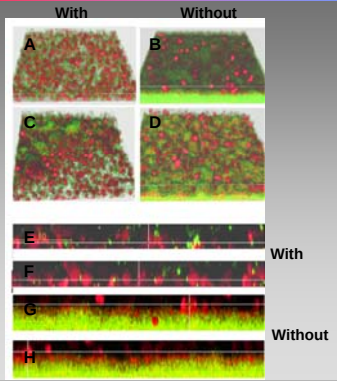
"The Battlefield" (diagram of a medical biofilm)



PMNs and *P. aeruginosa* biofilm

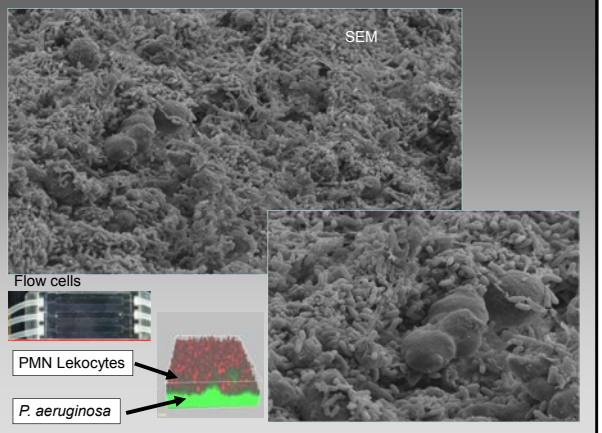


PMNs and Garlic treatment

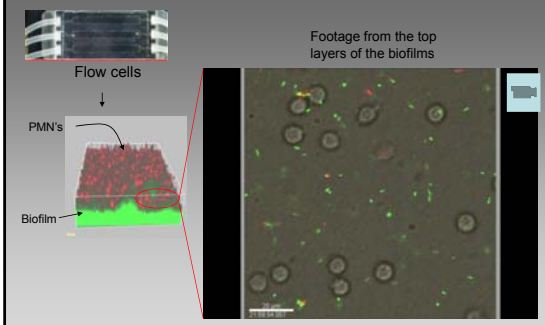


This suggests that the difference in clearing between a QS functional and a QS deficient strain is caused by:

- Virulence inhibition
- Enhanced activity of the PMN's

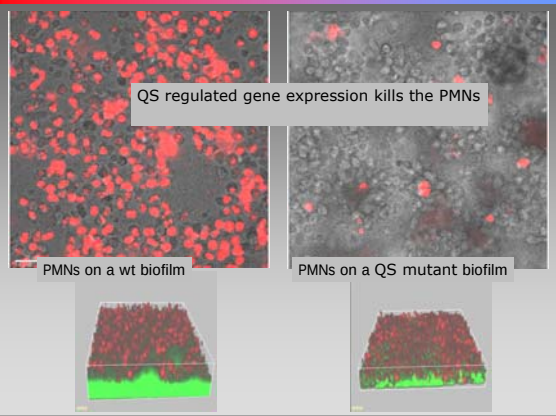


PMNs versus biofilm bacteria



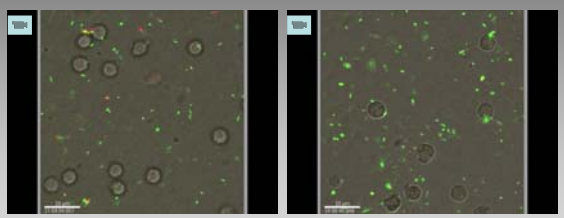
Bjarnsholt T., Jensen P.Ø., Burmølle M., Hentzer M., Haagensen J.A., Hougen H.P., Calum H., Madsen K.G., Moser C., Molin S., Hoiby N., Givskov M. 2005. *Pseudomonas aeruginosa* tolerance to tobramycin, hydrogen peroxide and polymorphonuclear leukocytes is quorum-sensing dependent. Microbiology.151:373-83.

PI live/dead stain of PMNs



Live from the Battlefield

O_2^- + hydroethidine → 2-hydroxyethidium (red fluorescence))
2-hydroxyethidium + DNA (increased red fluorescence)

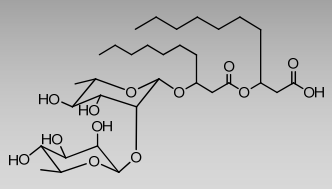


Wild type

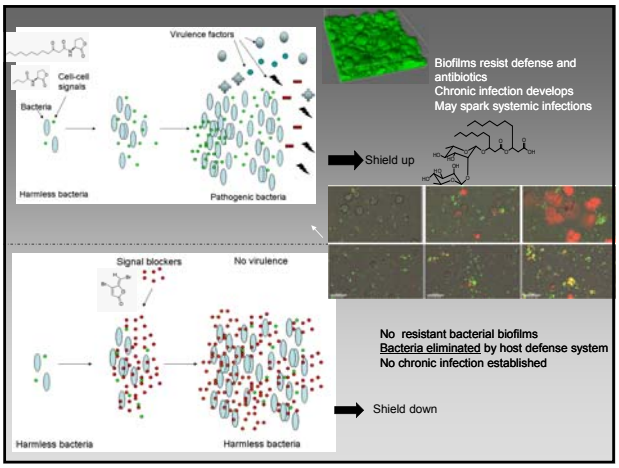
QS mutant (Δ*lasRrhlR*)

The shield against PMNs

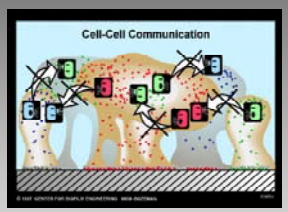
- We purified and identified the toxin to be **rhamnolipid**, 2-O-α-L-Rhamnopyranosyl- α -L-rhamnopyranosyl-β-hydroxydecanoyl- β -hydroxydecanoic acid.



The rhamnolipid production is controled by Quorum sensing (QS)



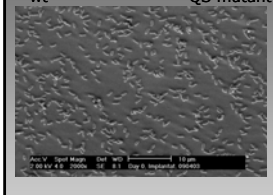
Clearing of the wild-type vs. the rhamnolipid mutant

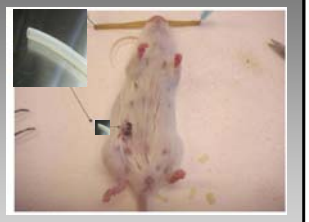


- We have earlier shown that a QS deficient *P. aeruginosa* is cleared faster than the wild-type in a *in vivo* lung model

The implant mouse model vers. 2

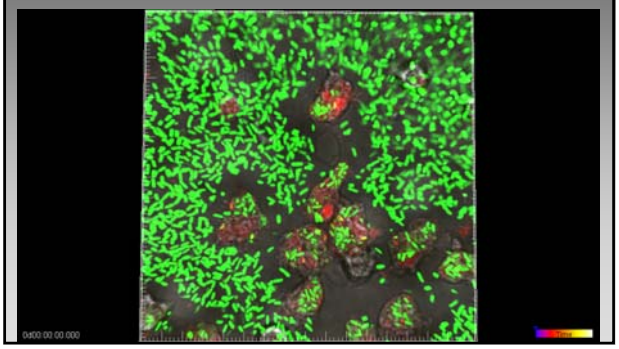
wt QS mutant



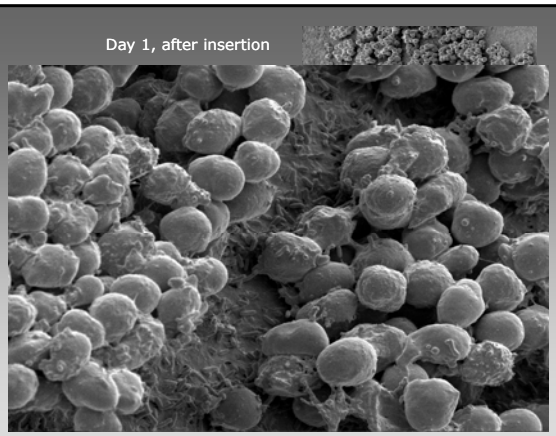


Day 0, P.a. coated silicone tube prior to insertion

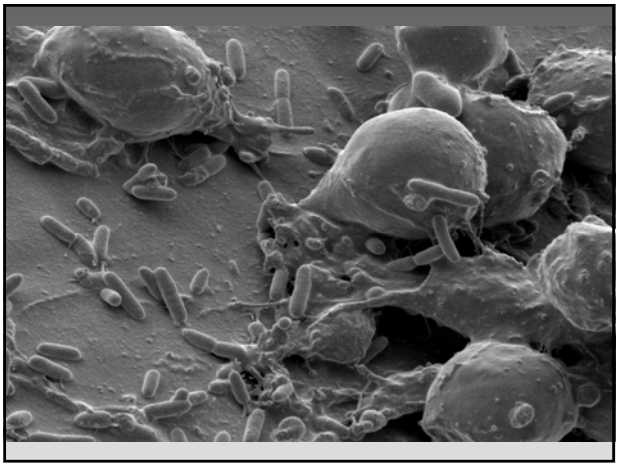
Initial showdown (in vitro)

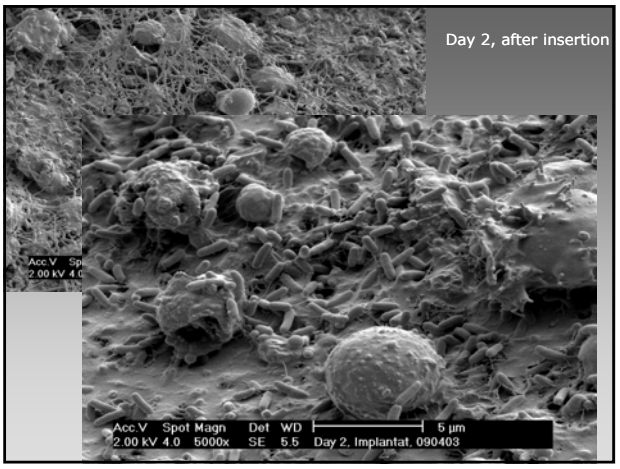


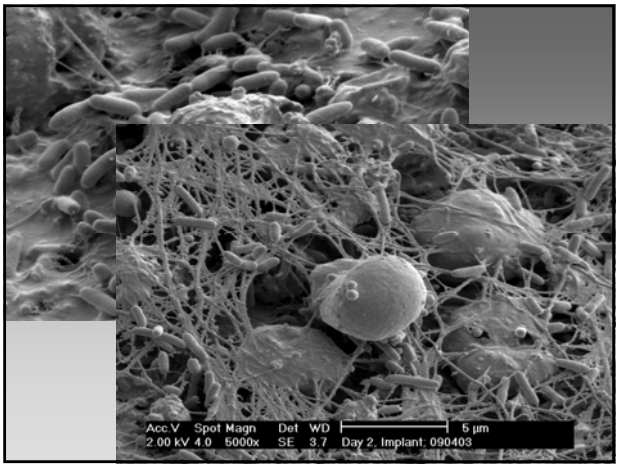
Day 1, after insertion

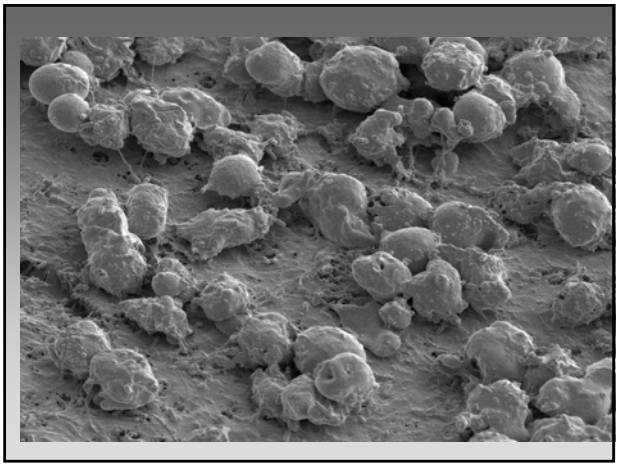


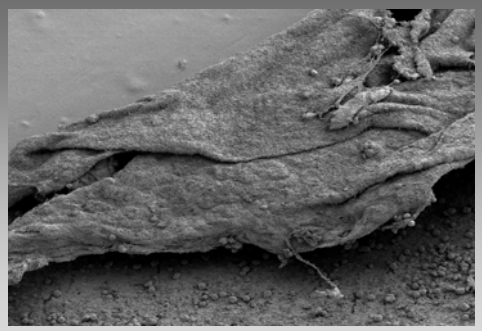
Bjarnsholt & Qvortrup



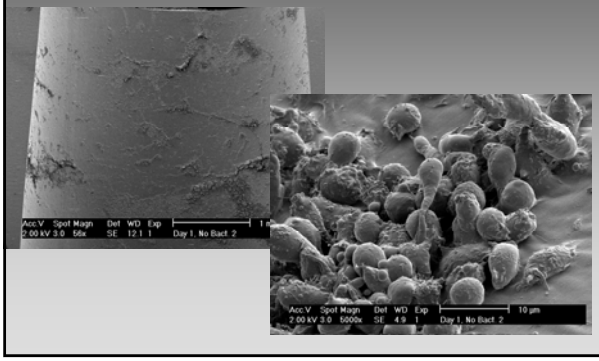

Day 2, after insertion
Acc.V Spot Magn Det WD 5 µm
2.00 kV 4.0 5000x SE 5.5 Day 2, Implantat. 090403


Acc.V Spot Magn Det WD 5 µm
2.00 kV 4.0 5000x SE 3.7 Day 2, Implant. 090403

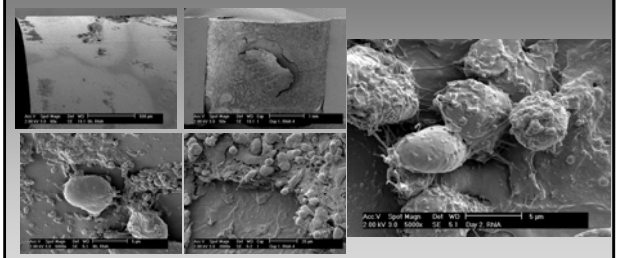




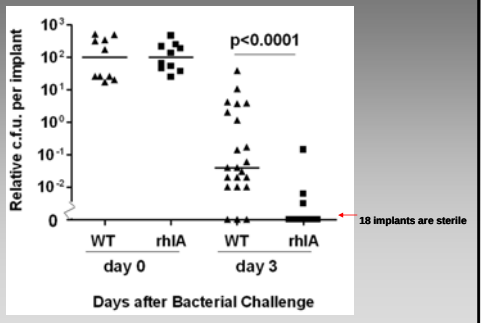
No bacteria, day 1



RhlA mutant



A Δ*rhlA* mutant is cleared rapidly from silicone implants



Resistance to QSI?

- Compounds capable of paralyzing QS systems do not affect any vital function of the bacterium and thus will not interfere with its growth.
- When growth is not affected, there is not a harsh selection pressure for the development of resistant bacteria
- Communities of helpful and beneficial bacteria present in the host (for example the gut flora) are not eliminated.

The hypothesis

- Blocking of QS create an adequate immune response which destroy only the bacteria and not the fragile surrounding tissue.
- We envision that a treatment based on QSI drugs and antibiotics will delay the chronic *P. aeruginosa* infection seen in CF
- Help to prolong the life of the CF patients.
- Enable clearance of bacterial biofilms from implants, chronic wounds and chronic otitis media etc.

However, is this clinical relevant

- QS regulation takes place the CF lung
- High amounts of free DNA possibly from PMNs
- High amount of necrotic PMNs in the chronic infected CF lung
- Biofilm mode of growth in the CF lung, chronic wounds, and chronic otitis media etc.
  - very tolerant

Signal analysis?

- The interaction between host and bacteria
- Initiation of the QS regulation
- Antimicrobial kinetics on biofilms

Collaborators

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Michael Givskov, Tim Holm Jacobsen, Louise D. Christensen, Maria Van Gennip, Morten Alhede, Oana Chiofu and Anne Kirstine Nielsen

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Niels Høiby, Peter Østrup Jensen, Claus Moser, Henrik Calum, Helle Krog Johansen, Lars Christophersen

Tacjana Pressler and Christine Rønne Hansen, Copenhagen CF Center
Klaus Kirketerp-Møller- Copenhagen Wound Healing Center, Bispebjerg Hospital
Claus Bøgelund Andersen- Dept. of Pathology, Rigshospitalet
Hans Petter Hougen- Inst. of Forensics., University of Copenhagen
Jette Pedersen- Bartholin Instituttet
Preben Homøe- Dept. of Otolaryngology, Head & Neck Surgery, Rigshospitalet
Thomas Sams – DTU Elektro

AdvanDx